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Study of Lipase Producing Bacterial Strains from Oil Contaminated Soil

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Abstract: Lipase producing bacterial strains were isolated from oil contaminated soils and grown on nutrient agar medium containing 1% (w/v) Tween-20. The isolate showing maximum activity was identified by following Berger's manual. Three bacterial strains such as *Bacillus* sp., *Staphylococcus* sp. and *Clostridium* sp. were isolated from oil contaminated soil sample. Different media parameters were optimized for maximal enzyme production. Peak lipase activity was observed glucose as carbon source, peptone as nitrogen source, at pH 7.0 and temperature at 40°C.

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INTRODUCTION

A lipase is an enzyme that catalyzes the hydrolysis of fats (lipids). Lipases are a subclass of the esterase. Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms. Hydrolytic enzymes like lipases furnish the greatest share in the industrial enzyme market. Lipases (Triacylglycerol, EC 3.1.1.3) are a major group of biocatalysts that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids. In wake of recent advancements in microbiology & biotechnology, lipases have emerged as key enzymes owing to their multifaceted properties which find use in a wide array of industrial applications. Lipase have been isolated and purified from fungi, yeast, bacteria, plant and animal sources but bacterial lipases are more economical and stable. Bacterial lipases are extensively used in food and dairy industry, cheese ripening, flavour enhancement, detergent industry, textile industry, for synthesis of biodegradable polymers or compounds, different trans-esterification reactions, cosmetic industry, in pulp and paper industry, in synthesis of biodiesel, and in pharmaceutical industries. The present investigation focused on isolation, screening and optimization of lipase producing bacterial strains from different parameters.

MATERIALS & METHODS:

Sample Collection: Sample was collected from oil contaminated soil of automobile garage area situated in Sector-6 Market, Bhilai, Chhattisgarh.

Isolation of Lipase Producing Bacteria: Soil sample has been serially diluted and plated on to the Nutrient Agar medium, pH 7.0 by spread plate method. Plates were incubated at 37°C for 48 hours. Pure cultures of the isolates were maintained on nutrient agar slants and were subcultured every 15 days.

Morphological and Biochemical Characterization of Lipase Producing Bacterial Strains:

The morphological and biochemical characterization of lipase producing bacterial isolates were done in accordance with the Bergey's manual of systematic bacteriology.

Screening of the Isolates for Lipase Activity: Lipolytic microorganisms were screened by qualitative plate assay method. Bacterial strains were gown on nutrient medium substrate containing Tween-20 agar plates and incubated at 37°C for 24 to 48 hours and zone were observed.

Lipase Production and Isolation: The composition of production medium used in this study was: (%w/v) peptone 0.2; $NH_4H_2PO_4$ 0.1; NaCl 0.25; $MgSO_4.7H_2O$ 0.04; $CaCl_2.2H_2O$ 0.04; olive oil 2.0 (v/v); pH 7.0; 1-2 drops Tween 20 as emulsifier. Overnight cultures were suspended in 5ml of sterile deionised water and used as the inoculum for pre culture to obtain an initial cell density to adjust the turbidity. Submerged microbial cultures were incubated in 500 ml Erlenmeyer flasks containing 100 ml of liquid medium on a rotary shaker (150 rpm) and incubated at 36°C. After 24 hours of incubation, the culture was centrifuged at 10,000 rpm for 20 min at 4°C and the cell free culture supernatant fluid was used as the sources of extracellular enzyme. The lipase activity in the supernatant was determined by the colorimetric method.

Optimization of Fermentation Conditions Time Course of Lipase Production: The time course of lipase production was studied in the enzyme production medium in shake flasks incubated for 60 hours. 5% inoculum was added to 50 ml of medium, in 500-ml Erlenmeyer flasks and incubated at 150 rpm on a rotary shaker, at 36°C, for 80 hrs. Samples were removed periodically at 8 hr interval and bacterial growths as well as lipase activity in the culture supernatant were determined.

Effect of the Medium pH and Incubation Temperature: The effect of pH and temperature of the fermentation medium for lipase production was performed by varying pH of the medium from 4 to 10 whereas the other parameters were unaltered. For selection of optimum temperature for the production of lipases, the temperatures varying from 20°C to 60°C were selected by keeping the remaining parameters same [7].

Effect of Carbon Sources: Effect of carbon source on the lipase production was analyzed by replacing the olive oil with different carbon sources Glucose, Sucrose, Starch, Lactose and Fructose at a concentration of (1% w/v) were added into the production medium in 500 ml Erlenmeyer flasks containing 100 ml of liquid medium on a rotary shaker (150 rpm) and incubated at 36°C for 24 hrs and the enzyme was assayed [7].

Effect of Nitrogen Sources: Effect of nitrogen sources on the lipase production was studied the nitrogen sources with Beef extract, casein, peptone, yeast extract and urea at a final concentration of 1 % (w/v) were added to the medium and incubated at 36°C for 24 hrs in a rotary shaker (150 rpm) [7].

RESULTS:

Screening and Morphological Identification of Lipase Producing Bacterial Strains: Soil sample was collected from automobile garage area showed high bacterial count. The colonies labeled as strain-1, 2 and strain-3 showed maximum zone observed when plated on nutrient agar medium containing Tween-20. Morphological and biochemical studies were done on these isolates. These bacterial strains were Gram positive. In accordance with the Bergey's manual of systematic bacteriology, the isolates were likely to be belonging to genus *Staphylococcus* sp., *Bacillus* sp. and *Clostridium* sp. as Bergey's manual system, and through biochemical characterization by Aneja (2003).

Effect of the Medium pH and Incubation Temperature: For selection of optimum temperature and pH for the production of lipases, the temperatures varying from 20 to 60°C and pH-3.0, 5.0, 7.0, 9.0 and 10.0 were selected by keeping the remaining parameters same. The study was conducted result have obtained maximum lipase activity was observed at pH-7.0 on 40°C by *Bacillus* sp. (4.22), then *Staphylococcus* sp. (2.32) then after *Clostridium* sp. (1.89).

Table 1: Biochemical Characterization of Three Bacterial Strains

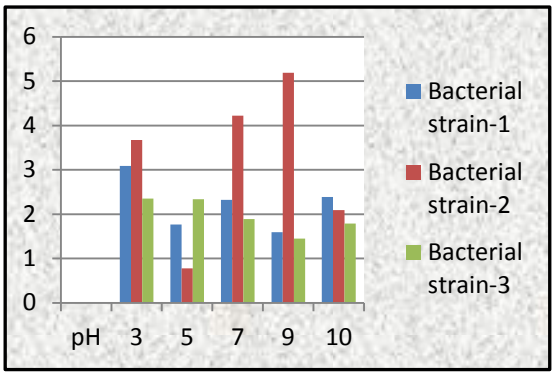
S. No.	Biochemical Characters		Strain-1		Strain-2		Strain-3	
1.	Gram's Staining		*Staphylococcus* +ve		*Bacillus* +ve		*Clostridium* +ve	
2.	Carbohydrate Fermentation							
	Glucose	Lactose	+ve	+ve	+ve	-ve	+ve	+ve
3.	Gelatin Hydrolysis		+ve		+ve		+ve	
4.	Amylase Hydrolysis		-ve		+ve		+ve	
5.	Catalase Activity		-ve		-ve		-ve	
6.	Lipase Production		+ve		+ve		+ve	
7.	Methyl-Red Reaction		+ve		-ve		+ve	
8.	Voges-Proskauer Reaction		+ve		+ve		+ve	
9.	Citrate Utilization		-ve		+ve		+ve	
10.	Indole Hydrolysis		-ve		-ve		-ve	
11.	TSIA Test		-ve		-ve		-ve	
12.	Urease Activity		-ve		-ve		-ve	
Identification of Bacterial Strains			***Staphylococcus* sp.**		***Bacillus* sp.**		***Clostridium* sp.**	

Table 2: Effect of Temperature on Lipase production by Three Strains (Optical Density in 540nm-20, 30, 40, 50, 60°C)

	Bacterial Strain-1	Bacterial Strain-2	Bacterial Strain-3
20	2.34	0.22	1.79
30	0.77	0.67	2.12
40	2.65	0.56	2.68
50	0.59	0.17	1.89
60	0.26	0.09	2.09

Table 3: Effect of pH on Lipase Production by Three Strains (Optical Density in 540nm)

S. No.	pH	Bacterial Strain-1	Bacterial Strain-2	Bacterial Strain-3
1.	3	3.09	3.67	2.35
2.	5	1.77	0.78	2.34
3.	7	2.32	4.22	1.89
4.	9	1.59	5.19	1.45
5.	10	2.39	2.09	1.79



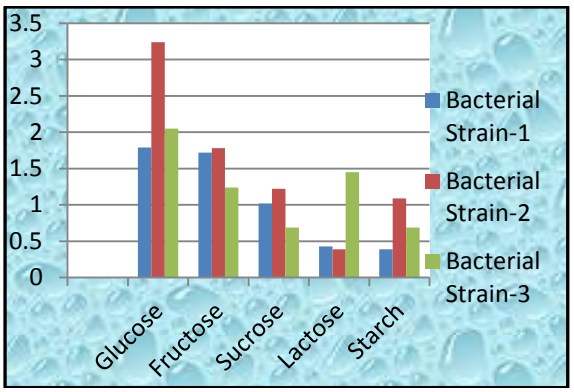
Graph 1: Effect of pH on Lipase Production by Three Strains (Optical Density in 540nm)

Effect of Carbon Source

In the present study it has been observed that lipase production was obtained maximum growth by using glucose medium as carbon source in *Bacillus* sp. then *Staphylococcus* sp. then *Clostridium* sp.

Table 4: Effect of Carbon Source on Lipase Production by Three Strains (Optical Density in 540nm)

S. No.	Carbon Source	Bacterial Strain-1	Bacterial Strain-2	Bacterial Strain-3
1.	Glucose	1.79	3.24	2.05
2.	Fructose	1.72	1.78	1.24
3.	Sucrose	1.02	1.22	0.69
4.	Lactose	0.43	0.39	1.45
5.	Starch	0.39	1.09	0.69

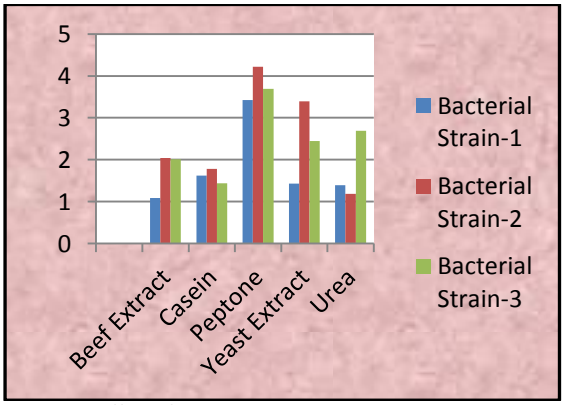


Graph 2: Effect of Carbon Source on Lipase Production by Three Strains (Optical Density in 540nm)

Effect of different Nitrogen Sources on lipase production: Optimization was carried out by using different organic nitrogen as nitrogen sources. Different nitrogen source used were Peptone, Beef extract and Yeast Extract, Urea, and Casein were added to the medium and incubated at 40°C for 24 hrs in a rotary shaker. In the present investigation peptone is a good medium of nitrogen source for lipase production because of *Bacillus* sp. is maximum growth yielding as a comparison of *Staphylococcus* sp. and *Clostridium* sp.

Table 5: Effect of Nitrogen Source on Lipase Production by Three Strains (Optical Density in 540nm)

S. No.	Nitrogen Source	Bacterial Strain-1	Bacterial Strain-2	Bacterial Strain-3
1.	Beef Extract	1.09	2.04	2.01
2.	Casein	1.62	1.78	1.44
3.	Peptone	3.42	4.22	3.69
4.	Yeast Extract	1.43	3.39	2.45
5.	Urea	1.39	1.19	2.69



Graph 3: Effect of Nitrogen Source on Lipase Production by Three Strains (Optical Density in 540nm)

1. Pure Culture of Bacterial Strains

Fig 1: Pure Culture of Bacterial Strain-1, Strain-2 and Strain-3

2. Gram's Staining of Bacterial Culture:

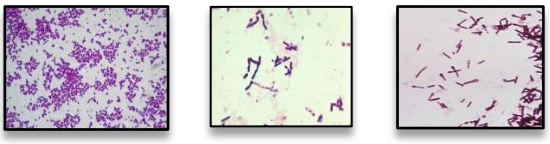


Fig 2: Bacterial Strain-1: + ve *Staphylococcus* sp.

Fig 3: Bacterial Strain-2: + ve *Bacillus* sp.

Fig 4: Bacterial Strain-3: + ve *Clostridium* sp.

Lipase Screening Activity:

Fig 5: Bacterial Strain- Lipase (Tween-20) - (1) Positive, (2) Positive, (3) Positive

Lipase Activity of Bacterial Crude Extracts Against Tween-20 Agar Plate (Agar Well Diffusion Method):

Fig 6: Bacterial Strain- Crude Lipase Activity - (1), (2), (3) showing growth in Tween-20 Agar Medium

CONCLUSION

I have concluded that, bacterial lipases are enzymes having important market value. Into these three isolates *Bacillus* sp. has shown the highest production of extracellular lipase enzyme. In optimization studies highest lipase production were shown in glucose as good carbon source, peptone as nitrogen source at pH-7.0 and 40°C temperature range. The extracellular lipase enzyme can be further purified and used indifferent industrial applications.

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